AD_____

Award Number: W81XWH-11-1-0414

TITLE: Prevention and Treatment of Noise-Induced Tinnitus

PRINCIPAL INVESTIGATOR: Dr. Richard A. Altschuler

CONTRACTING ORGANIZATION: The University of Michigan
Ann Arbor, MI 48109

REPORT DATE: July 2012

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-07-2012	ANNUAL	1 Jul 2011- 30 Jun 2012

4. TITLE AND SUBTITLE
Prevention and Treatment of Noise-Induced Tinnitus

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0414

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Richard Altschuler

E-Mail: shuler@umich.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Michigan

Ann Arbor, Michigan 48109-1274

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Results indicate that a small arms fire – like noise will induce tinnitus in

Results from the first year of studies indicate that a 2 ½ minute exposure to a small arms fire – like noise will induce tinnitus in some but not all of the noise-exposed rats. The noise exposure generates a hearing loss (as measured by the auditory brain stem response) ranging from mild to large that correlates with the amount of sensory cell (hair cell) loss induced by the noise. There can also be a loss of some of the auditory nerve terminals in the cochlear nucleus. Studies are now assessing the amount of loss of inner hair cell – auditory nerve connections and looking for a correlation between each type of loss and the appearance of tinnitus. Studies are also now examining if anti-oxidant and anti-excitotoxicity therapeutics prior to the noise exposure will prevent the losses and reduce the incidence of tinnitus.

15. SUBJECT TERMS
Tinnitus, Noise, Protection, Cochlea, Auditory, Excitotoxicity, Oxidative Stress, Anti-oxidants

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	10	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

I. INTRODUCTION:

A high incidence of Tinnitus can result as an outcome of battlefield noise and this has become a major health concern for military, reducing the ability to redeploy, reducing the quality of life of those affected and increasing health care costs. The proposed studies have two goals. One goal is "PREVENTION", to develop treatments that if provided prior to, during or shortly after a noise exposure will prevent tinnitus from being induced. These studies are based on a hypothesis that treatments must protect both sensory cells (hair cells) and connections to the auditory nerve from being lost in order to be effective. Studies test for correlation of both types of losses with progression of tinnitus and test the influence of drugs that prevent or reduce the loss. The second goal is "TREATMENT", to develop treatments that can reduce or eliminate tinnitus after it has occurred. These second group of studies are based on a hypothesis that once tinnitus has become established the focus of treatment must move from the cochlea to the central auditory system and studies will test therapies that reduce neuronal excitability to reduce or eliminate the percept of tinnitus.

II. BODY:

A. STATEMENT OF WORK – There are four tasks to be accomplished over the three years of studies:

TASK 1: a) Test the hypothesis that the manifestation of tinnitus from a military relevant noise exposure will correlate with the co-appearance and amount of outer hair cell loss and inner hair cell – auditory nerve (IHC-AN) connection loss. **b)** Test the hypothesis that the manifestation of tinnitus from a military relevant noise exposure will correlate with the amount of loss of auditory nerve terminals in the ventral cochlear nucleus.

TASK 2: Test the hypothesis that therapeutic interventions to prevent noise-induced tinnitus must preserve BOTH outer hair cells and IHC AN connections to be effective.

TASK 3: Test the hypothesis that decreases in inhibitory synaptic strength and changes in neuronal excitability will be greatest in animals with noise-induced chronic tinnitus and less in animals receiving comparable noise exposure but not developing tinnitus**.**

TASK 4: Test the hypothesis that use of therapy to increase inhibitory synaptic strength (e.g. sarcosine) or correct changes ion channel expressions that regulate excitability (e.g. retigabine) will reduce or eliminate chronic tinnitus.

B. KEY RESEARCH ACCOMPLISHMENTS:

Research Accomplishments for TASK 1: a) Test the hypothesis that the manifestation of tinnitus from a military relevant noise exposure will correlate with the co-appearance of outer hair cell loss and IHC-AN connection loss; **b)** Test the hypothesis that the manifestation of tinnitus from a military relevant noise exposure will correlate with the loss of auditory nerve connections on spherical bushy cells in the anteroventral cochlear nucleus (AVCN).

- We have tested three noise exposure conditions to mimic the effects of small arms fire in the military. There is a range of hearing loss and loss sensory cells (inner and outer hair cells) for each of the three exposures (as is seen in people). We have chosen an exposure condition (152dB in 50 bursts over 2 ½ minutes) that gives a moderate to large loss of outer hair cells (Figure One) and a loss of hearing as occurs in up to a third of the military exposed to such noise. Figure One in the Supporting Data section show hair cell loss following such exposure in the rat model in a representative animal. Figure Two in the Supporting Data section shows hearing loss immediately following the noise exposure as measured by the auditory brain stem response (ABR).

- The next component of this task was to determine the incidence of tinnitus in the rats following the small arms fire - like noise-exposures. In order to test our hypothesis on whether there is a correlation of manifestation of tinnitus with the degree of loss of both outer hair cells IHC-AN connections, the noise

conditions must generate both rats developing tinnitus and noise-exposed rats that do not have tinnitus. Our results are finding that approximately 60% of noise-exposed rats show evidence of tinnitus, consistent with our preliminary results and sufficient to allow for the therapeutic interventions that have already begun in year 1 in TASK 2 (see below). Figure Three in the Supporting Data section gives examples of reduced gap detection in two representative animals, providing indication of tinnitus.

- We have generated a normative data base for Inner Hair Cell – Auditory Nerve Connections per inner hair cell in five different regions of the rat cochlea based on quantitative assessment of confocal images of CTBP2 immunostaining in the rat cochlea (Figure Four). This normative data base is now being used to determine changes in the number of connections under our year one experimental conditions.

- We have now assessing the input-output function of the Auditory Brainstem Response (ABR) growth function, as a measure of auditory nerve function and auditory nerve loss that can be assessed over the course of the "in-life" phase of the studies. We have generated a normative data base that we are now using as a baseline to quantitate changes under our experimental conditions. An example of normal I/O functions can be viewed in Figures Five and Six in the Supporting Data section. We are now working to semi-automate this assessment to increase efficiency in years 2 and 3 as we analyze therapeutic interventions.

- We are in the process of completing the normative data base(s) of VGLUT1, VAT and VGAT immunostaining in the rat AVCN and DCN that will allow assessment of changes under our experimental conditions. Initial results indicate some loss of VGLUT1 immunolabeled auditory nerve terminals in the ventral cochlear nucleus ipsilateral to the noise exposure.

Research Accomplishments for TASK 2 – Test the hypothesis that therapeutic interventions to prevent or reduce the Aim 1 changes will prevent or reduce the appearance of noise-induced tinnitus, testing anti-apoptotic intervention (ACEMg) to reduce hair cell loss and anti-excitotoxic intervention (memantine and piribedil) to reduce loss of IHC-AN connections.

These studies are underway and analysis of hair cell loss, CTBP2 immunostaining (IHC-AN connections) from animals with different treatment is in progress, but has not reached the point where there is a sufficient number of animals with and without tinnitus under the different treatment conditions for enough power to have a report on results.

Research Accomplishments for TASK 3: Test the hypothesis that the loss of AN terminals (marked by VGLUT1 immunolabel) on neurons in the AVCN and DCN will be followed by increases in inappropriate excitatory influences (with VGLUT2 immunolabel marking non-auditory nerve glutamatergic terminals and VAT marking cholinergic terminals) and decreases in inhibitory amino acid terminals (marked by VGAT), changing the balance in excitatory – inhibitory influence towards reduced inhibitory synaptic strength.

- As mentioned for TASK 1, we are in the process of completing the normative data base(s) of VGLUT1, VAT and VGAT immunostaining in the rat AVCN and DCN, that will allow assessment of changes under our experimental conditions. We have acquired the cochlear nucleus from animals with and without noise-induced tinnitus that will be processed and assessed in Year 2 for this determination.

Research Accomplishments for TASK 4 – Test the hypothesis that treatments which reverse the increased excitability induced by changes elucidated in Aim 3 will reduce or eliminate noise-induced chronic tinnitus.

- Task 4 studies will begin in year 2

C. REPORTABLE OUTCOMES:

Data bases:

We have generated a normative data base of **Inner Hair Cell – Auditory Nerve (IHC-AN) Connections** per inner hair cell in different regions of the Sprague Dawley rat cochlea based on quantitative assessment of confocal images of CTBP2 immunostaining in the rat cochlea. This normative data base is now being used as the base-line to determine changes in (IHC-AN) connections under the experimental conditions.

We have generated a normative data base of the number of **Inner Hair Cells (IHCs) and Outer Hair Cells (OHCs)** along the cochlear spiral for the normal Sprague Dawley rat cochlea for use in our cytocochleogram program. This normative data base is now being used as the base-line to determine changes in IHCs and OHCs under the experimental conditions.

We have generated a normative data base of **Auditory Brainstem Response (ABR) growth functions** (Input – Output functions) at different frequencies for the Sprague Dawley rat. This normative data base is now being used as the base-line to determine changes under the experimental conditions.

D. CONCLUSION:

The research completed in Year One clearly shows that small arms fire – like noises will result in tinnitus in some but not all of the noise-exposed rats, with an incidence of approximately 55%. Rats without noise exposure do not show tinnitus. All of the rats show some hair cell loss and hearing loss from this exposure. There is also a noise-induced loss of inner hair cell – auditory nerve synapses. As more rats are run through study and assessed we will be able to determine if there is a relationship between the amount of hair cell loss and the amount of loss if inner hair cell – auditory nerve. The future assessments will examine central auditory changes as well, as see if these might correlate with tinnitus. We will also begin examining therapeutic interventions that influence hair cell loss and loss of connections and determine if these influence the incidence of tinnitus.

SUPPORTING DATA:

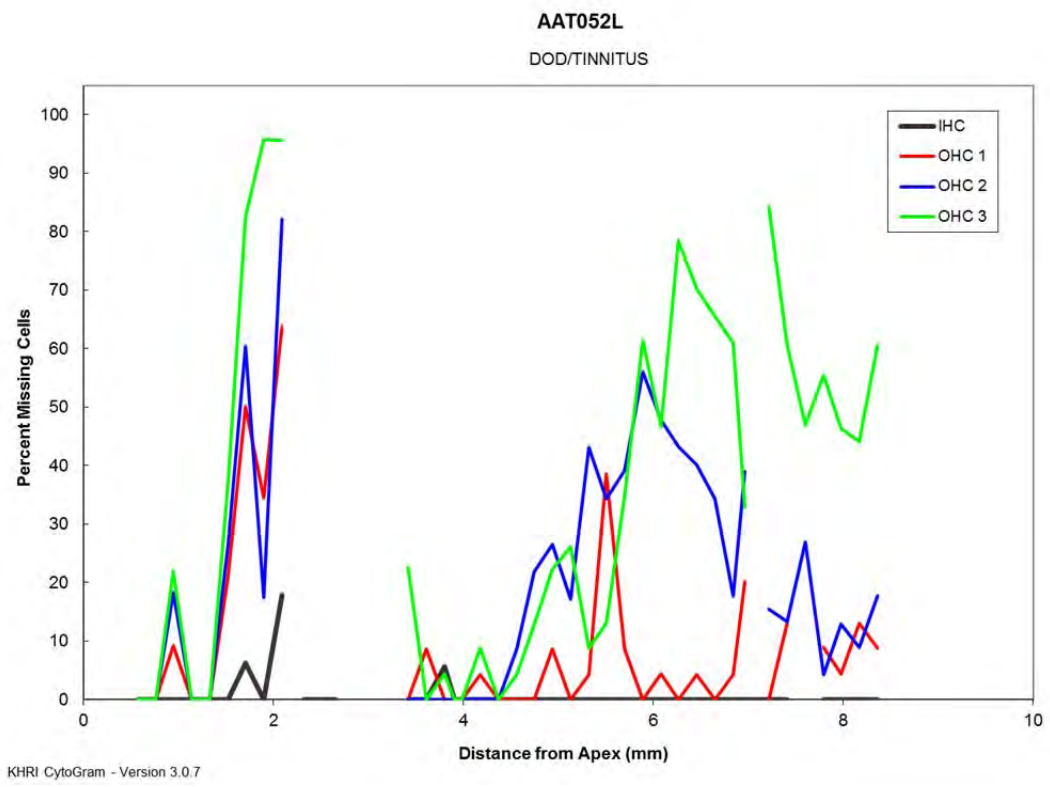


FIGURE ONE: A cytocochleogram showing hair cell loss following a small arms fire – like noise in a representative animal. The number of missing Inner Hair Cells (IHC – thick black line) and Outer Hair Cells (OHC) in row 1 (red line), row 2 (blue line) and row 3 (green line) is plotted from the apex (left) to the base (right) of the cochlear spiral following a brief small arms fire – like noise exposure of 152 dB. There is a small region of inner hair cell about 2 mm from the apex with moderate outer hair cell loss in all rows in that region as well. There is a larger region (4 – 8 mm from the apex) of Outer Hair Cell loss that is mild to moderate in the third row and less in the first row.

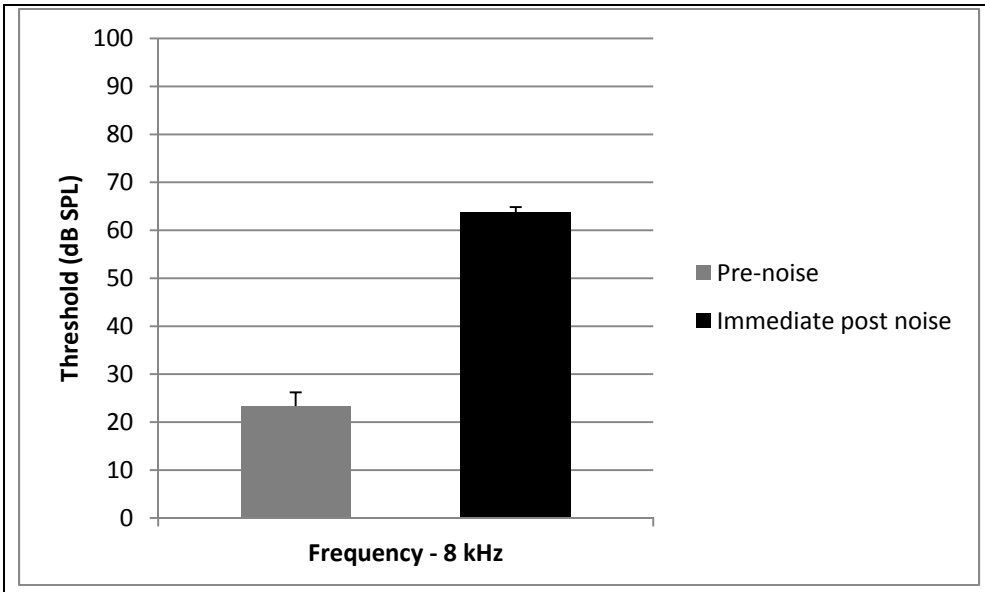
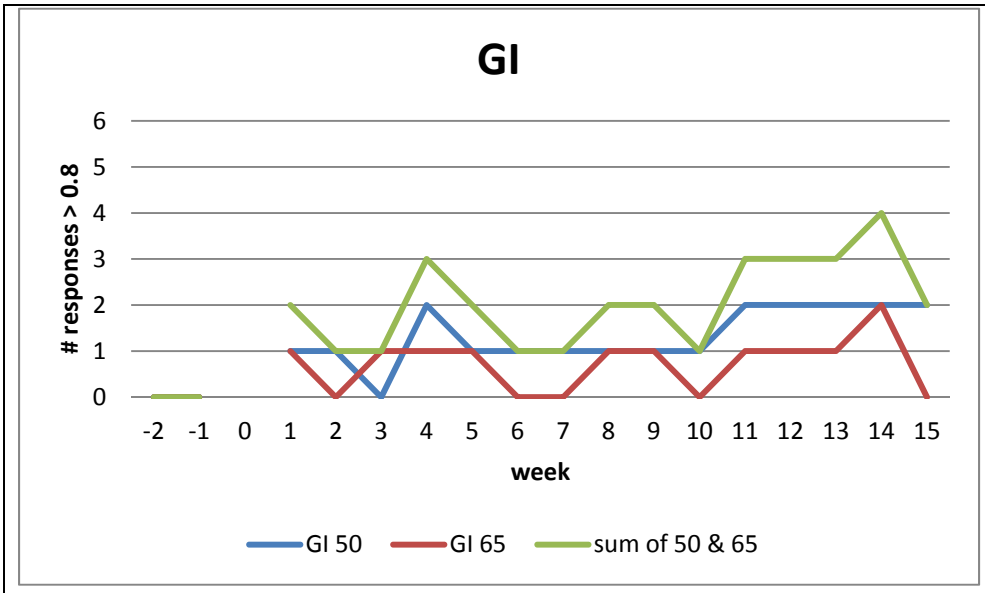


FIGURE TWO: A graph showing the mean of auditory brain stem response (ABR) thresholds from rats prior to noise exposure (grey bar on right) of 152 dB impulse noise compared to the ABR threshold post-noise (black bar on right) showing an increase in threshold (threshold shift) of 40.3 dB that was significant, $p<0.0001$.



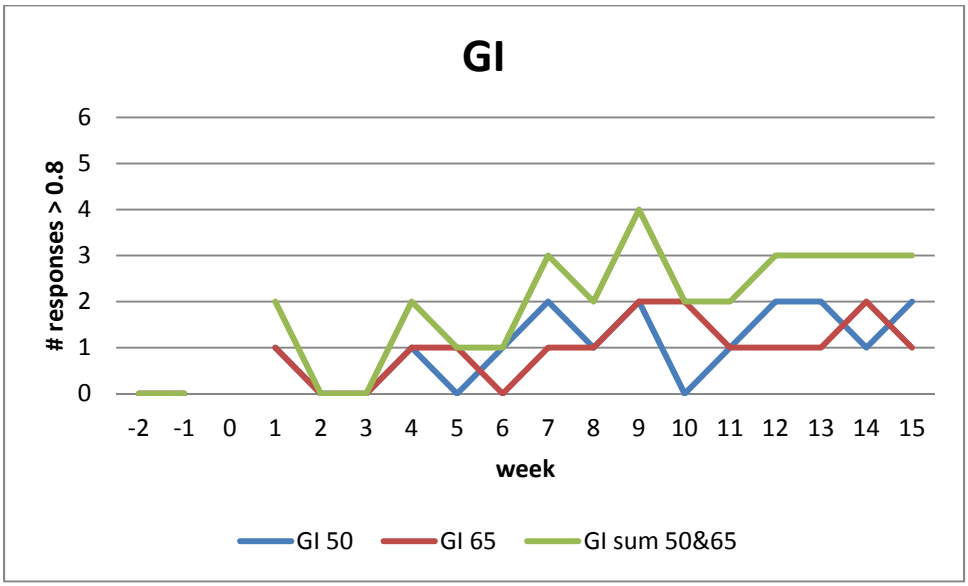


FIGURE THREE: A measure of Gap Inhibition (GI) of the acoustic startle reflex over time, first measured for the two weeks before noise exposure (-2, -1), with noise exposure then at "O" and then followed for 15 weeks following the noise. Tinnitus is marked by reduced gap inhibition. This figure shows two rats that are representative of the animals that develop tinnitus. They show normal GI before the noise (no responses less than 0.8), with reduced gap detection (higher number in the graph) appearing immediately after the noise, then returning to closer to normal before reappearing at 3-4 weeks following the noise exposure and becoming most consistent at 10-11 weeks after the noise.

FIGURE FOUR

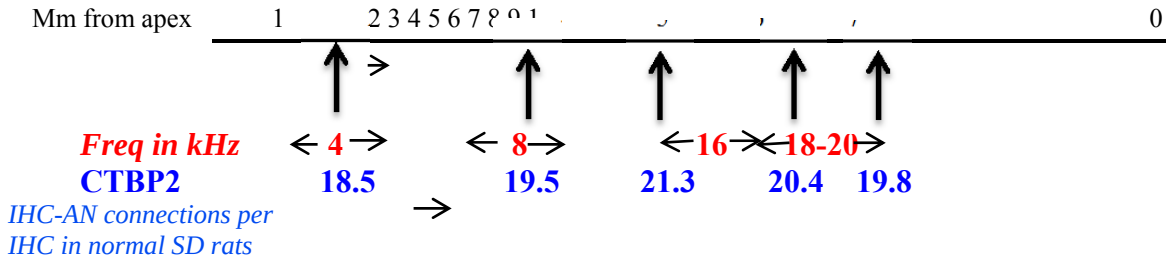


Figure Four – The mean number of CTBP2 immunolabeled synaptic ribbons per inner hair cells in five regions of the cochlear spiral in normal Sprague Dawley rats, as a marker for the number of inner hair cell – auditory nerve (IHC-AN) connections per inner hair cell in different regions. The number of mm from the apex is marker above, the location of the four frequencies being tested in the auditory brain stem response measure in labeled in red. The "normative" number of CTBP2 ribbons (and IHC-AN connections) per inner hair cell is given in blue – the arrows point to the locations where the assessment was made.

FIGURE FIVE

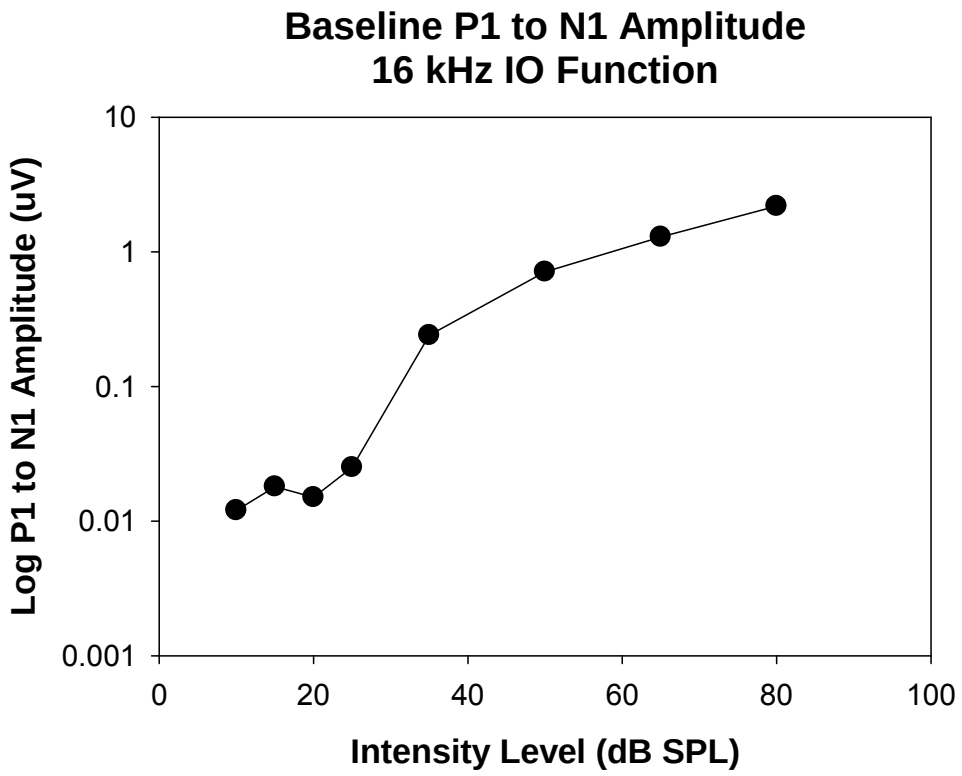


Figure Five – The peak-to-peak amplitude of P1 to N1 of the ABR is shown as a function of the intensity of a 16 kHz tone burst. It results from the synchronous neural contribution of the auditory nerve to these peaks and reflects the numbers of CTBP2 ribbons (and IHC-AN connections) at the inner hair cell.

Figure Six – ABR Input/Output function (assessing the peak-to-peak amplitude at increasing intensity) means from six normal rats